

REMARKS

This amendment is filed along with a Request for Continued Examination, as the amendment is expected to require further consideration and/or search. This application has been amended in a manner to place it in condition for allowance.

**Status of the Claims**

Claim 15 is amended. Support for the amendment to the claims may be found, for example, at pages 8-9 of the specification.

Claims 15-34 are pending in this application.

Claims 15-19, 23-25 and 29-32 were examined on the merits and stand rejected.

Claims 20-22, 26-28, 33, and 34 were withdrawn as non-elected subject matter.

**Information Disclosure Statement**

Applicants acknowledge with appreciation the consideration of JP 2002-537849.

However, as to JP 2000-83656, which was cited on the IDS of December 13, 2005, it is respectfully submitted that applicants are not required to provide an English translation of JP 2000-83656, as an English version of the International Search Report citing this document has been provided. The Examiner's attention is respectfully directed to MPEP 609.04(a) III, in

particular, the second paragraph. For the Examiner's convenience, however, an English Abstract of JP 2000-83656 is submitted with this amendment with additional copies of the International Search Report which cited JP 2000-83656.

Thus, consideration of JP 2000-83656 is respectfully requested.

### **Priority**

As to the requirement of an English translation of a foreign priority document, Applicant's are not required to provide an English translation pursuant to MPEP 201.15.

### **Claim Rejections-35 USC §102**

Claims 15, 18, 23, and 24 stand rejected under 35 U.S.C. § 102(b) as anticipated by PARK (Bone, 1999, 24: 549-554) as evidenced by LECOEUR (Biomaterials, 1997, 18: 989-993). This rejection is respectfully traversed for the reasons that follow.

The amendment to claim 15 clearly differentiates the preadipocytes of the present invention from those of PARK, as described in further detail below:

#### **Preadipocytes of the present invention**

Fig. 1 shows micrographs of the fraction after filtration of step (i), and it is clear that the fraction only contains unilocular adipocytes. Mature adipocytes which are to

be dedifferentiated into preadipocytes in the present invention are unilocular adipocytes alone.

Preadipocytes of the present invention have no lipid droplets and already expressing an early marker of osteoblast, myoblast or adipocyte.

Cells according to PARK

Cells contained in a suspended fraction after centrifugation of the bone marrow samples are used as "adipocytes" by PARK. Though bone marrow contains various cells (for example, osteoblast, bone marrow stromal cells), no treatment with collagenase or filtration is done in PARK, which means that PARK should not obtain a fraction which only contains unilocular adipocytes, unlike the present invention.

The position of the Official Action is that the PARK cells are not contaminated with bone marrow stromal cells, etc. To the contrary, however, PARK states (Page 549, left column, lines 3-5) "The object of the present study was: i) to isolate relatively pure populations of adipocytes from human bone marrow". That is, PARK acknowledges there are some contaminations by the term, "relatively".

At Page 553, left column, lines 33-47, PARK further states,

"We have found that, as may be expected, preadipocytes and mature adipocytes are abundant in the floating fat layer of

human bone marrow. In this layer, the original starting material used for this study, a large number of single adipocytes are present, although some of the adipocytes are associated in conglomerates with fibroblastic cells\*. This observation is similar to a previous report by Blazsek et al., which defined the culture of the hematoma, a multicellular functional unit from human bone marrow. In our short-term culture system we observed adherence of the multicellular units\*\* and a large number of single adipocytes. The presence of adipocytes in such short-term cultures was never observed in parallel cultures of bone marrow fibroblastic cells derived from the marrow cell pellet. This result provides evidence that the cells present in the floating low-density layer of human bone marrow are mainly adipocytes and preadipocytes\*\*\*." (Emphasis added.)

\*Some adipocytes derived from bone marrow are contaminated with fibroblastic cells.

\*\*Multicellular units are observed.

\*\*\*PARK says that the cells are mainly adipocytes and preadipocytes, that is, mainly a mixture of adipocytes and preadipocytes.

Further, "preadipocytes" of PARK are simply fibroblast-like cells, which are different from "preadipocytes" of the present invention. The present preadipocytes are obtained by inducing dedifferentiation by passage culture from fibroblast-like adipocytes having no lipid droplets, the fibroblast-like

adipocytes being obtained by ceiling culture. The fibroblast cells which PARK refers to as "preadipocytes" are not dedifferentiated by the above steps of the present invention. EP Examiner admitted the difference.

In view of the above comparison, PARK uses different cells for inducing differentiation from the preadipocytes of the present invention, PARK cannot anticipate the claimed invention.

Therefore, withdrawal of the rejection of the 102(b) rejection of claims 15, 18, 23, and 24 over PARK is requested.

**Claim Rejections-35 USC §103**

Claims 15-18, 23, 24, 29, and 30 stand rejected under 35 U.S.C. § 103(a) as obvious over PARK taken with LECOEUR in view of SUGIHARA (Differentiation, 1986, 31:42-49).

Claims 15-19, 23-25, and 29-32 were rejected under 35 U.S.C. § 103(a) as obvious over PARK and LECOEUR and SUGIHARA in view of ROSS (Science, 2000, 289:950-953), BENNETT (J. Biol. Chem., June 7, 2002, 277:30998-31004) and RANDO (J. Cell Biol., 1994, 125:1275-1287).

These rejections are respectfully traversed and are discussed together below in view of their common references.

As mentioned above, PARK fails to disclose the claimed invention, as the cells and the preadipocytes of PARK differ from the present invention.

SUGIHARA cannot remedy the shortcomings of PARK, however, for reference purposes.

The fibroblast-like adipocytes of SUGIHARA are obtained by ceiling culture like the present invention. However, SUGIHARA fails to disclose or suggest obtaining fibroblast-like cells having no lipid droplets, or inducing dedifferentiation by passage culture of the fibroblast-like adipocytes having no lipid droplets.

Preadipocytes of the present invention are established as preadipocyte cell line having no lipid droplets which express an early marker of osteoblast, myoblast or adipocyte during the passage culture.

On the other hand, the fibroblast-like adipocytes of SUGIHARA have lipid droplets, become mature adipocytes by contact inhibition, and synthesize large lipid droplets under the influence of insulin. This is because the adipocytes of SUGIHARA are not dedifferentiated, unlike the preadipocytes of the present invention. The Examiner's attention is respectfully directed to the differences listed in Table 1.

Preadipocytes of the present invention were obtained in a similar way as disclosed in JP 2000-83656, and have similar characteristics as those of the cells obtained in JP 2000-83656.

Table 1. Comparison of the preadipocyte cell line of the present invention and Sugihara's cell

		Preadipocytes of the present invention		Sugihara's cell	
Established cell		preadipocyte		Fibroblast-like cell	
Characteristics	Form	Fibroblast		Multilocular cell, fibroblast-like adipocyte	
	Lipid droplet	×		○	
	Capability of dedifferentiation	○		○	
	Passage culture	○	Retains functions as preadipocyte after passage culture for a long period	–	
	Mature by contact inhibition	×	Does not become mature by contact inhibition	○	Matures into unilocular adipocyte by contact inhibition
Redifferentiation by differentiation inducing agent (DEX, IBM)		○	Becomes unilocular adipocyte by using differentiation inducing agent. The activity value against G3PDH increases, and lipid droplets become to be observed, and stained by oil red O	×	Already differentiated
Influence of insulin		×	Not affected until being differentiated into adipocyte by differentiation inducing agent	○	Because already differentiated, affected and synthesize large lipid droplets.
Expression of early markers	PPAR $\gamma$ adipocyte	○		–	Not identified. Assumed to express markers.
	Cbfa1 osteoblast	○		–	Not identified. Not assumed to express markers.
	Myf5 myoblast	○		–	Not identified. Not assumed to express markers.

The position of the Official Action alleges that the cells of SUGIHARA are presumed to express the same early markers as the preadipocytes of the present invention because Sugihara's cells are obtained by ceiling culture.

However, as shown above, the fibroblast-like adipocytes according to SUGIHARA retain the characteristics of adipocytes, and it is very unlikely that they express an early marker of osteoblast and myoblast. It is acknowledged that in a stage where a cell is committed to be an adipocyte and differentiated, the expression of group of genes which is specific to the differentiation of other cells are suppressed strictly.

Though the preadipocyte of the present invention is a cell in a stage of preadipocyte which has been dedifferentiated from adipocyte, it expresses an early marker of osteoblast and myoblast. The preadipocyte of the present invention is very unique and shows unexpected results.

Further, the inventors have confirmed that the adipocyte produced according to the present method does not express Glu4, which is a later and terminal marker of adipocytes (See Evidence 1 and 2 in the appendix). Glu4 has a function of taking glucose into cytoplasm, glucose being a raw material for fat synthesis in response to insulin. Also preadipocyte of the present invention is not affected by insulin by itself. This further supports that preadipocyte of the present invention is

not at the terminal state in the differentiation into an adipocyte, but in the state where it loses functions as an adipocyte and is dedifferentiated.

As mentioned above, preadipocytes of the present invention are different from the cells of PARK or fibroblast-like adipocytes of SUGIHARA. Even if one were to replace the "preadipocyte" of PARK with the cell of SUGIHARA cell, preadipocyte of the present invention could not be obtained.

It would not have been expected that dedifferentiation into osteoblast occurs if PARK and LECOEUR are combined. Further preadipocyte of the present invention (FERM BP-0864, claim 14) is not obtained by the method of SUGIHARA, but by dedifferentiation of adipocyte, and has unexpected characteristics. Thus the present invention is unobvious over SUGIHARA.

As to the specific rejection of claims 15-19, 23-25 and 29-32 over PARK, LECOEUR and SUGIHARA in view of ROSS, BENNETT and RANDO, this alleges that they disclose that:

- adipocytes and myoblasts originate from preadipocytes,
- preadipocyte expresses Wnt10b receptor at high level,  
and
- signaling by Wnt10b is required for commitment to the myocyte lineage, and the inhibition of Wnt10b signaling in preadipocytes and myoblasts induces adipogenesis.

The Official Action concludes that a skilled man in the art would have known that treating preadipocytes with a myoblast

differentiation medium comprising Wnt10b would result in their transdifferentiation to myoblasts.

However, preadipocytes of the present invention are different from the cells of either PARK or SUGIHARA, and they are not derived from mouse embryo. Wnt10b is not added in inducing differentiation of the preadipocytes to myoblasts.

It is well known that it is more difficult to differentiate mesenchymal stem cells into myoblasts than into osteoblasts, adipocytes and chondrocytes, and that there have been few successes in obtaining myoblasts. Even with 10T1/2 cells which is the only mesenchymal stem cells capable of differentiating into skeletal muscle, transcription controlling region of skeletal muscle is highly methylated compared to myoblasts, and the differentiation frequency is very low. Further, C2C12 is the only cell line which can be used for the study of differentiation of skeletal muscle. The only other optional to obtain myoblast is to use muscle satellite cells which exist on myofibrils of muscle tissues (See Evidence 3 to 5 of the appendix).

In view of the above, the one skilled in the art would not have expect that myoblasts can be easily obtained from preadipocytes of the present invention based on ROSS where Wnt10b was used.

ROSS knocked out Wnt, and used over expression technique to obtain myoblasts from preadipocytes. If it were

obvious to differentiate preadipocytes of the present invention into myoblasts based on the above references alone including ROSS, as the suggested by the Official Action, it would have been obvious to obtain various differentiated cells from ES-cell-like ips cells only because various cells are obtained from ES cells.

In the present invention Wnt10b was not used in differentiation of adipocytes into myoblasts, and specific medium system was found in order to produce skeletal muscle from cells originated from adipocytes.

Therefore, the present invention is not rendered obvious over cited references, and withdrawal of the rejection is respectfully requested.

### **Conclusion**

In view of the amendment to the claims and the foregoing remarks, this application is in condition for allowance at the time of the next Official Action. Allowance and passage to issue on that basis is respectfully requested.

Should there be any matters that need to be resolved in the present application, the Examiner is respectfully requested to contact the undersigned at the telephone number listed below.

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any

overpayment to our credit card which is being paid online simultaneously herewith for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

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**APPENDIX:**

The Appendix includes the following items:

- English Abstract of JP 2000-83656
- JP 2000-83656
- The International Search Report for PCT/JP2004/007322 which  
explains the relevance of JP 2000-83656

**-EVIDENCE:**

1. Yagi et al., Biochem. Biophys. Res. Commun., 321,  
pp. 967-974 (2004).
2. Nobusue et al., Cell Tissue Res., 332, pp. 435-446  
(2008).
3. Brian P. et al., Developmental Biology, 177, pp.  
490-503 (1996).
4. Stephan F. et al., Cell, 38, pp. 791-800 (1984).
5. Timothy J. et al., Development, 109, pp. 139-148  
(1990).